

drewwang

FILE 'CAPLUS' ENTERED AT 10:47:55 ON 03 JAN 2001  
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FILE 'EMBASE' ENTERED AT 10:47:55 ON 03 JAN 2001  
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FILE 'MEDLINE' ENTERED AT 10:47:55 ON 03 JAN 2001

FILE 'SCISEARCH' ENTERED AT 10:47:55 ON 03 JAN 2001  
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=> s u6 and ribozyme#

L1 89 U6 AND RIBOZYME#

=> s l1 and localiz?

L2 14 L1 AND LOCALIZ?

=> dup rem l2

PROCESSING COMPLETED FOR L2  
L3 5 DUP REM L2 (9 DUPLICATES REMOVED)

=> d 1- ibib abs

YOU HAVE REQUESTED DATA FROM 5 ANSWERS - CONTINUE? Y/(N):y

L3 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1  
ACCESSION NUMBER: 1997:46429 CAPLUS  
DOCUMENT NUMBER: 126:71160  
TITLE: The expression cassette determines the functional activity of **ribozymes** in mammalian cells by controlling their intracellular **localization**  
AUTHOR(S): Bertrand, Edouard; Castanotto, Daniela; Zhou, Chen; Carbonnelle, Cecile; Lee, Nan Sook; Good, Paul; Chatterjee, Saswati; Grange, Thierry; Pictet, Raymond;  
Kohn, Donald; Engelke, David; Rossi, John J.  
CORPORATE SOURCE: Dep. Molecular Biol., Beckman Research Institute City of Hope, Duarte, CA, 91010, USA  
SOURCE: RNA (1997), 3(1), 75-88  
CODEN: RNARFU; ISSN: 1355-8382  
PUBLISHER: Cambridge University Press  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB In order to better understand the influence of RNA transcript context on RNA **localization** and catalytic RNA efficacy in vivo, we have constructed and characterized several expression cassettes useful for transcribing short RNAs with well defined 5' and 3' appended flanking sequences. These cassettes contain promoter sequences from the human U1 snRNA, U6 snRNA, or tRNAMet genes, fused to various processing/stabilizing sequences. The levels of expression and the sub-cellular **localization** of the resulting RNAs were detd. and

compared with those obtained from Pol II promoters normally linked to mRNA prodn., which include a cap and polyadenylation signal. The tRNA, U1, and U6 transcripts were nuclear in localization and expressed at the highest levels, while the std. Pol II promoted transcripts were cytoplasmic and present at lower levels. The ability of these cassettes to confer **ribozyme** activity in vivo was tested with two assays. First, an SIV-growth hormone reporter gene was transiently transfected into human embryonic kidney cells expressing an anti-SIV **ribozyme**. Second, cultured T lymphocytes expressing an anti-HIV **ribozyme** were challenged with HIV. In both cases, the **ribozymes** were effective only when expressed as capped, polyadenylated RNAs transcribed from Pol II cassettes that generate a cytoplasmically **localized ribozyme** that facilitates co-localization with its target. The inability of the other cassettes to support **ribozyme**-mediated inhibitory activity against their cytoplasmic target is very likely due to the resulting nuclear **localization** of these **ribozymes**. Thus, the **ribozyme** expression cassette det. its intracellular **localization** and, hence, its corresponding functional activity.

L3 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2  
ACCESSION NUMBER: 1997:82132 CAPLUS  
DOCUMENT NUMBER: 126:194873  
TITLE: Expression of small, therapeutic RNAs in human cell nuclei  
AUTHOR(S): Good, P. D.; Krikos, A. J.; Li, S. X. L.; Bertrand, E.; Lee, N. S.; Giver, L.; Ellington, A.; Zaia, J. A.;  
Rossi, J. J.; Engelke, D. R.  
CORPORATE SOURCE: Dep. Biol. Chem., Univ. Michigan, Ann Arbor, MI, USA  
SOURCE: Gene Ther. (1997), 4(1), 45-54  
CODEN: GETHEC; ISSN: 0969-7128  
PUBLISHER: Stockton  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Effective intracellular expression of small RNA therapeutics depends on a no. of factors. The RNA, whether antisense, **ribozyme**, or RNA aptamer, must be efficiently transcribed, stabilized against rapid degrdn., folded correctly, and directed to the part of the cell where it can be most effective. To overcome a no. of these problems we have been testing expression cassettes based on the human tRNA<sup>met</sup> and U6 snRNA promoters, in which transcripts encoding small RNA inserts are protected against attack from the 3' end. Transient expression in cultured cells results in 103-2 .times. 107 full-length transcripts per cell, depending partially on the promoter construct used but also on the nature of the insert RNA. 5'-gamma-Phosphate methylation (capping) depended, as expected, on the inclusion of specific U6 snRNA sequences from positions +19 to +27. In situ **localization** of the transcripts shows that both tRNA and U6 promoter transcripts give primarily punctate nuclear patterns, and that capping of transcripts is not required for nuclear retention. Several different insert RNAs directed against HIV-1 were tested by cotransfection with HIV-1 provirus and assay for subsequent viral reverse transcriptase prodn. These include antisense RNA, hairpin and hammerhead **ribozymes**, and RNA ligands (aptamers) for Tat and Rev RNA-binding proteins. Results show that Rev-binding RNAs efficiently block HIV-1 gene expression, whereas other RNAs have little or no effect when expressed in these cassettes.

L3 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2001 ACS  
ACCESSION NUMBER: 1996:491417 CAPLUS  
DOCUMENT NUMBER: 125:136221  
TITLE: Differences in the interaction of Escherichia coli RNase P RNA with tRNAs containing a short or a long

extra arm  
 AUTHOR(S): Gaur, Rajesh K.; Hanne, Andreas; Conrad, Frank; Kahle,  
 Dietmar; Krupp, Guido  
 CORPORATE SOURCE: Inst. Allgemeine Mikrobiologie, Christian-Albrechts-  
 Univ., Kiel, D-241 18, Germany  
 SOURCE: RNA (1996), 2(7), 674-681  
 CODEN: RNARFU; ISSN: 1355-8382  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB The phosphorothioate footprinting technique was applied to the  
 investigation of phosphate moieties in tRNA substrates involved in  
 interactions with M1 RNA, the catalytic subunit of Escherichia coli RNase  
 P. In general agreement with previous data, all affected sites were  
 localized in acceptor stem and T arm. But the analyzed examples  
 for class I (Saccharomyces cerevisiae pre-tRNAPhe with short variable  
 arm)  
 and class II tRNAs (E. coli pre-tRNATyr with large variable arm) revealed  
 substantial differences. In the complex with pre-tRNAPhe, protection was  
 obsd. at U55, C56, and G57, along the top of the T loop in the tertiary  
 structure, whereas in pre-tRNATyr, the protected positions were G57, A58,  
 and A59, at the bottom of the T loop. These differences suggest that the  
 size of the variable arm affects the spatial arrangement of the T arm,  
 providing a possible explanation for the discrepancy in reports about the  
 D arm requirement in truncated tRNA substrates for eukaryotic RNase P  
 enzymes. Enhanced reactivities were found near the junction of acceptor  
 and T stem (U6, 7, 8 in pre-tRNAPhe and G7, U63, U64 in  
 pre-tRNATyr). This indicates a partial unfolding of the tRNA structure  
 upon complex formation with RNase P RNA.

L3 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3  
 ACCESSION NUMBER: 1996:54465 CAPLUS  
 DOCUMENT NUMBER: 124:137591  
 TITLE: Efficient hammerhead ribozyme-mediated  
 cleavage of the structured hepatitis B virus  
 encapsidation signal in vitro and in cell extracts,  
 but not in intact cells  
 AUTHOR(S): Beck, Juergen; Nassal, Michael  
 CORPORATE SOURCE: Zentrum Molekulare Biologie, Univ. Heidelberg,  
 Heidelberg, D-69120, Germany  
 SOURCE: Nucleic Acids Res. (1995), 23(24), 4954-62  
 CODEN: NARHAD; ISSN: 0305-1048  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB Hepatitis B virus (HBV), the causative agent of B-type hepatitis in man,  
 is a small enveloped DNA virus that replicates through reverse  
 transcription of an RNA intermediate, the terminally redundant RNA  
 pregenome. An essential highly conserved cis-element present twice on  
 this  
 RNA is the encapsidation signal .epsilon., a stem-loop structure that is  
 crit. for pregenome packaging and reverse transcription. .epsilon. Is  
 hence an attractive target for antiviral therapy. Its structure,  
 however,  
 is a potential obstacle to antivirals whose action depends on  
 hybridization, e.g. ribozymes. Here we demonstrate effective in  
 vitro cleavage inside .epsilon. by hammerhead ribozymes contg.  
 flanking sequences complementary to an adjacent less structured region.  
 Upon co-transfection with a HBV expression construct corresponding  
 ribozymes embedded in a U6 snRNA context led to a  
 significant, though modest, redn. in the steady-state level of HBV  
 pregenomes. Inactive ribozyme mutants revealed that antisense  
 effects contributed substantially to this redn., however, efficient  
 .epsilon. cleavage by the intracellularly expressed ribozymes  
 was obsd. in Mg2+-supplemented cell lysates. Artificial HBV pregenomes  
 carrying the ribozymes in cis and model RNAs lacking all HBV  
 sequences except .epsilon. exhibited essentially the same behavior.

Hence, neither the absence of co-localization of **ribozyme** and target nor a viral component, but rather a cellular factor(s), is responsible for the strikingly different **ribozyme** activities inside cells and in cellular exts.

L3 ANSWER 5 OF 5 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.  
ACCESSION NUMBER: 92102726 EMBASE  
DOCUMENT NUMBER: 1992102726  
TITLE: Association of U6 snRNA with the 5'-splice site region of pre-mRNA in the spliceosome.  
AUTHOR: Sawa H.; Shimura Y.  
CORPORATE SOURCE: Department of Biophysics, Faculty of Science, Kyoto University, Kyoto 606, Japan  
SOURCE: Genes and Development, (1992) 6/2 (244-254).  
ISSN: 0890-9369 CODEN: GEDEEP  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 022 Human Genetics  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB U6 snRNA is one of the five RNA species required for splicing of nuclear pre-mRNAs. High conservation of its sequence has led to the hypothesis that U6 snRNA plays a catalytic role in splicing. If this is the case, U6 snRNA should be **localized** close to sites where the splicing reaction occurs. However, this has never been demonstrated. Here, we have shown that U6 snRNA is cross-linked to the 5'-splice site region of pre-mRNA by UV irradiation during the in vitro splicing reaction. We have also detected the cross-link of U6 snRNA and the region around the branchpoint of the intron lariat. The results show that U6 snRNA is present near the splice sites in the splicing reaction and support the idea that U6 snRNA is a catalytic element in the spliceosome.

=> d his

(FILE 'HOME' ENTERED AT 10:47:40 ON 03 JAN 2001)

FILE 'CAPLUS, EMBASE, MEDLINE, SCISEARCH' ENTERED AT 10:47:55 ON 03 JAN 2001

L1 89 S U6 AND RIBOZYME#  
L2 14 S L1 AND LOCALIZ?  
L3 5 DUP REM L2 (9 DUPLICATES REMOVED)

=> s l1 and hiv

L4 24 L1 AND HIV

=> dup rem l4

PROCESSING COMPLETED FOR L4

L5 8 DUP REM L4 (16 DUPLICATES REMOVED)

=> d l- ibib abs

YOU HAVE REQUESTED DATA FROM 8 ANSWERS - CONTINUE? Y/(N):y

L5 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2001 ACS  
ACCESSION NUMBER: 2000:802344 CAPLUS  
DOCUMENT NUMBER: 133:359781  
TITLE: Improved accumulation and activity of **ribozymes** expressed from RNA-based RNA polymerase III promoters  
INVENTOR(S): Thompson, James D.

PATENT ASSIGNEE(S): Ribozyne Pharmaceuticals, Inc., USA  
 SOURCE: U.S., 46 pp., Cont.-in-part of U.S. Ser. No. 293,520,  
 abandoned.  
 CODEN: USXXAM  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 30  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6146886	A	20001114	US 1995-512861	19950807
US 5902880	A	19990511	US 1994-337608	19941110
CA 2207593	AA	19960620	CA 1995-2207593	19951122
WO 9618736	A2	19960620	WO 1995-US15516	19951122
WO 9618736	A3	19960919		
W: AU, CA, JP, MX				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 799313	A2	19971008	EP 1995-942944	19951122
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE				
AU 9644120	A1	19960703	AU 1996-44120	19951130
AU 9939188	A1	19990916	AU 1999-39188	19990713
PRIORITY APPLN. INFO.:			US 1994-293520	19940819
			US 1994-337608	19941110
			US 1994-354920	19941213
			US 1994-363253	19941223
			US 1994-363254	19941223
			US 1995-390850	19950217
			US 1995-426124	19950420
			US 1995-432874	19950502
			US 1995-434509	19950504
			AU 1995-26422	19950518
			US 1995-951	19950707
			US 1995-974	19950707
			US 1995-512861	19950807
			US 1995-541365	19951005
			WO 1995-US15516	19951122

AB A transcribed non-naturally occurring RNA mol. is provided comprising a desired RNA mol., wherein the 3' region of the RNA is able to base-pair with at least 8 bases at the 5' terminus of the same RNA mol. RNA polymerase III (polIII) transcripts are abundant in all cells.

Therefore,

pol III promoters may be ideal for expressing high levels of exogenous RNAs, such as antisense RNAs, decoy RNAs, and **ribozymes**, in many different cell types. Improved accumulation of recombinant RNAs

expressed

from a human tRNA<sup>i</sup>Met-derived polIII promoter >100-fold by modifying the 3'-terminus of the transcripts to hybridize to the 5'-terminus. This terminal duplex includes the 8-nucleotide leader sequence present in the primary wild-type Meti-tRNA transcript that is normally removed during processing to the mature tRNA. Expression of an anti-HIV **ribozyme** was analyzed in cells stably transduced with retroviral vectors encoding polIII transcription units contg. this modification. High accumulation of recombinant polIII **ribozyme** transcripts was obsd. in all cell lines tested. Due to the enhanced transcript accumulation, **ribozyme** cleavage activity was readily detectable in total RNA extd. from stably transduced human T cell lines. One polIII transcription unit, termed 'TRZ', was optimized further for **ribozyme** cleavage activity. U6 small nuclear RNA- and adenovirus VAI-based polIII promoter systems are also described. The improved polIII transcription units reported here may be useful for expressing a variety of functional and therapeutic RNAs.

REFERENCE COUNT:

24

REFERENCE(S): (1) Adeniyi-Jones; Nucleic Acids Research 1984, V12, P1101 CAPLUS

(2) Anon; WO 8911539 1989 CAPLUS  
(3) Anon; WO 9013641 1990 CAPLUS  
(4) Anon; WO 9207065 1992 CAPLUS  
(5) Anon; WO 9311253 1993 CAPLUS  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1  
ACCESSION NUMBER: 2000:697876 CAPLUS  
TITLE: Rev-binding aptamer and CMV promoter act as decoys to inhibit HIV replication  
AUTHOR(S): Konopka, K.; Lee, N. S.; Rossi, J.; Duzgunes, N.  
CORPORATE SOURCE: School of Dentistry, Department of Microbiology, University of the Pacific, San Francisco, CA, 94115, USA  
SOURCE: Gene (2000), 255(2), 235-244  
CODEN: GENED6; ISSN: 0378-1119  
PUBLISHER: Elsevier Science B.V.  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB We examd. whether the antiviral effect of an HIV-1 Rev-binding aptamer [RBE(apt)] could be enhanced by a **ribozyme** directed against the HIV-1 env gene, and whether the antiviral activity was affected by different promoters. The efficacy of the aptamer and **ribozyme** DNAs was tested in HeLa cells co-transfected with the HIV-1 proviral clones, HXB.DELTA.Bgl or pNL4-3, using transferrin-lipoplexes. The RBE(apt) and anti-env **ribozyme** genes were inserted into the pTZU6+27 plasmid, or constructed under the control of the human cytomegalovirus (CMV) or Rous sarcoma virus (RSV) promoters. The parental vector plasmids were used as controls. Co-transfection of the pTZU6+27 RBE(apt) plasmid with HXB.DELTA.Bgl, or pNL4-3, at a wt. ratio of 5:1, inhibited p24 prodn. by 70 and 45%, resp. The RSV RBE(apt) plasmid co-transfected with either HIV clone, at the same wt. ratio, reduced viral prodn. by 88%. The addn. of the anti-env **ribozyme** to the RSV RBE(apt) did not enhance its antiviral activity. When the constructs were under the control of the

CMV promoter, the expression of the HIV plasmids was very low and was independent of the presence of the RBE(apt). Thus, the effect of the RBE(apt) was strongly dependent on the promoter of the tested construct. The anti-HIV activity of the CMV RBE(apt) construct was non-specific, because co-transfection with either pCMV.SPORT-.beta.gal or pCMVlacZ significantly suppressed HIV prodn. from the HIV proviral clones. The redn. in p24 could not be attributed to the non-specific toxicity of the transfection procedure. Transfection of acutely HIV-infected HeLa-CD4 cells with pCMV.SPORT-.beta.gal reduced the p24 level by 35%, while the expression of the U6 RBE(apt) did not affect p24 prodn. The suppression of HIV prodn. from the HIV proviral clones by the CMV promoter constructs in the co-transfection assays may be explained by competition for transcription factors (TFs) between HIV and CMV promoters. This observation points to the potential for misleading results in co-transfections involving CMV constructs and HIV.

REFERENCE COUNT: 30  
REFERENCE(S): (2) Adam, G; Gene 1996, V178, P25 CAPLUS  
(3) Bertrand, E; RNA 1997, V3, P75 CAPLUS  
(4) Boshart, M; Cell 1985, V41, P521 CAPLUS  
(5) Clark, P; Curr Opin Mol Therapeut 1999, V1, P158 CAPLUS  
(6) Doll, R; Gene Ther 1996, V3, P437 CAPLUS  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 3 OF 8 SCISEARCH COPYRIGHT 2001 ISI (R)  
ACCESSION NUMBER: 2000:623827 SCISEARCH  
THE GENUINE ARTICLE: 342HJ  
TITLE: RNA polymerase III-driven expression cassettes in human gene therapy

AUTHOR: Medina M F C (Reprint); Joshi S  
CORPORATE SOURCE: UNIV TORONTO, FAC MED, DEPT MED GENET & MICROBIOL,  
TORONTO, ON M5S 3E2, CANADA (Reprint)  
COUNTRY OF AUTHOR: CANADA  
SOURCE: CURRENT OPINION IN MOLECULAR THERAPEUTICS, (OCT 1999)  
Vol.

1, No. 5, pp. 580-594.  
Publisher: PHARMAPRESS LTD, MIDDLESEX HOUSE, 34-42  
CLEVELAND ST, LONDON W1P 6LB, ENGLAND.  
ISSN: 1464-8431.

DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 87

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB tRNAs, U6 small nuclear RNA and adenovirus virus associated  
RNAs are transcribed by RNA polymerase III. These RNAs are expressed in  
high levels and possess increased stability due to their small, compact  
size. Promoters expressing these RNAs have therefore been used to express  
a variety of interfering RNAs, including **ribozymes**, antisense  
RNAs and sense or decoy RNAs. Intracellular expression and activity of  
RNA  
polymerase III-driven interfering RNAs have been demonstrated in several  
studies. This review will summarize the design and application of RNA  
polymerase III-driven expression cassettes in human gene therapy using  
retroviral vectors as the primary method of gene delivery.

L5 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2

ACCESSION NUMBER: 1998:422577 CAPLUS

DOCUMENT NUMBER: 129:171080

TITLE: Virion encapsidation of tRNA<sup>3</sup>Lys-**ribozyme**  
chimeric RNAs inhibits HIV infection

AUTHOR(S): Westaway, Shawn K.; Cagnon, Laurence; Chang, Zongli;  
Li, Shirley; Li, Haitang; Larson, Garry P.; Zaia,

John

A.; Rossi, John J.

CORPORATE SOURCE: Dep. Mol. Biol., Beckman Res. Inst. of the City of  
Hope, Duarte, CA, 91010-3011, USA

SOURCE: Antisense Nucleic Acid Drug Dev. (1998), 8(3),  
185-197

CODEN: ANADF5; ISSN: 1087-2906

PUBLISHER: Mary Ann Liebert, Inc.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Retroviruses require a specific host cellular tRNA primer for initiation  
of first-strand DNA synthesis. This primer is bound by viral proteins  
and

copackaged into virions. We have exploited this property in the design  
and testing of an antiviral **ribozyme** fused to tRNA<sup>3</sup>Lys, the  
primer used for lentiviral replication, including human immunodeficiency  
virus (HIV-1 and HIV-2). The chimera consists of  
tRNA<sup>3</sup>Lys covalently attached to a hammerhead **ribozyme**, which is  
targeted to the region immediately upstream of the primer binding site of  
the HIV-1 genome. The tRNA-**ribozyme** chimeric  
transcript is catalytically active in vitro and is efficiently bound by  
HIV reverse transcriptase with an affinity similar to that of  
tRNA<sup>3</sup>Lys. We have expressed the chimeric RNAs from either the tRNA<sup>3</sup>Lys  
intragenic RNA polymerase III promoter or from a human U6 snRNA  
promoter. The U6 promoter results in up to 10-fold enhanced  
expression of the tRNA-**ribozyme**. Most importantly, the  
tRNA<sup>3</sup>Lys-**ribozymes** are encapsidated in HIV-1 virions  
such that they are effective in substantially reducing the level of  
infectious virus produced from cells cotransfected with HIV-1  
proviral DNA. These results demonstrate the feasibility of using this  
novel strategy to reduce HIV infectivity and more generally  
indicate the potential power of using the retroviral primer tRNAs as

tools

for expressing and delivering **ribozymes** and other antiretroviral RNAs to the virion capsid.

L5 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3  
ACCESSION NUMBER: 1998:204015 CAPLUS  
DOCUMENT NUMBER: 129:80  
TITLE: A simple assay system for examination of the inhibitory potential in vivo of decoy RNAs, **ribozymes** and other drugs by measuring the Tat-mediated transcription of a fusion gene composed of the long terminal repeat of HIV-1 and a gene for luciferase  
AUTHOR(S): Koseki, Shiori; Ohkawa, Jun; Yamamoto, Rika; Takebe, Yutaka; Taira, Kazunari  
CORPORATE SOURCE: MITI, National Institute of Bioscience and Human Technology, 1-1 Higashi, Tsukuba Science City, 305, Japan  
SOURCE: J. Controlled Release (1998), 53(1-3), 159-173  
CODEN: JCREEC; ISSN: 0168-3659  
PUBLISHER: Elsevier Science B.V.  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Nucleic acid-based drugs, including antisense RNA and DNA, **ribozymes** and decoys appear to have potential for the suppression of the expression of specific genes. To allow the examn. of the potential of such agents in vivo as anti-HIV drugs in std. labs., where facilities for handling live virions are not available, we constructed a simple assay system (HIV-1 model) that allows measurement of the extent of inhibition of Tat-mediated transcription of HIV-1 by nucleic acid-based drugs and other agents. In cells that harbor a stable chimeric long terminal repeat (LTR)-Luc construct (a fusion gene consisting of the LTR of HIV-1 and the gene for luciferase), total luciferase activity in an aliquot of cell lysate is dose- and promoter-dependent on transfection with a Tat expression plasmid, reflecting the character of the LTR promoter of HIV. When HeLa cells were co-transfected with the Tat expression plasmid and another plasmid that encoded the U6 promoter or the promoter of the gene for tRNAVal linked to the trans-activating response (TAR) sequence, total luciferase activity was inhibited by 60 or 40%, resp. The inhibition was also dependent on the dose of the TAR expression plasmid. These results demonstrate the usefulness of this simple assay system for detection of the efficacy of a decoy RNA or a **ribozyme** in vivo, without a requirement for HIV-infected cells, by measurement of luciferase activity in vitro.

L5 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 4  
ACCESSION NUMBER: 1997:46429 CAPLUS  
DOCUMENT NUMBER: 126:71160  
TITLE: The expression cassette determines the functional activity of **ribozymes** in mammalian cells by controlling their intracellular localization  
AUTHOR(S): Bertrand, Edouard; Castanotto, Daniela; Zhou, Chen; Carbonnelle, Cecile; Lee, Nan Sook; Good, Paul; Chatterjee, Saswati; Grange, Thierry; Pictet, Raymond;  
Kohn, Donald; Engelke, David; Rossi, John J.  
CORPORATE SOURCE: Dep. Molecular Biol., Beckman Research Institute City of Hope, Duarte, CA, 91010, USA  
SOURCE: RNA (1997), 3(1), 75-88  
CODEN: RNARFU; ISSN: 1355-8382  
PUBLISHER: Cambridge University Press  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB In order to better understand the influence of RNA transcript context on RNA localization and catalytic RNA efficacy in vivo, we have constructed



and characterized several expression cassettes useful for transcribing short RNAs with well defined 5' and 3' appended flanking sequences.

These

cassettes contain promoter sequences from the human U1 snRNA, U6 snRNA, or tRNAMet genes, fused to various processing/stabilizing sequences. The levels of expression and the sub-cellular localization of the resulting RNAs were detd. and compared with those obtained from Pol

II

promoters normally linked to mRNA prodn., which include a cap and polyadenylation signal. The tRNA, U1, and U6 transcripts were nuclear in localization and expressed at the highest levels, while the std. Pol II promoted transcripts were cytoplasmic and present at lower levels. The ability of these cassettes to confer **ribozyme** activity in vivo was tested with two assays. First, an SIV-growth

hormone

reporter gene was transiently transfected into human embryonic kidney cells expressing an anti-SIV **ribozyme**. Second, cultured T lymphocytes expressing an anti-HIV **ribozyme** were challenged with HIV. In both cases, the **ribozymes** were effective only when expressed as capped, polyadenylated RNAs transcribed from Pol II cassettes that generate a cytoplasmically localized **ribozyme** that facilitates co-localization with its target. The inability of the other cassettes to support **ribozyme**-mediated inhibitory activity against their cytoplasmic target is very likely due to the resulting nuclear localization of these **ribozymes**. Thus, the **ribozyme** expression cassette det. its intracellular localization and, hence, its corresponding functional activity.

L5 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2001 ACS

DUPLICATE 5

ACCESSION NUMBER: 1997:82132 CAPLUS

DOCUMENT NUMBER: 126:194873

TITLE: Expression of small, therapeutic RNAs in human cell nuclei

AUTHOR(S): Good, P. D.; Krikos, A. J.; Li, S. X. L.; Bertrand, E.; Lee, N. S.; Giver, L.; Ellington, A.; Zaia, J.

A.;

Rossi, J. J.; Engelke, D. R.

CORPORATE SOURCE: Dep. Biol. Chem., Univ. Michigan, Ann Arbor, MI, USA

SOURCE: Gene Ther. (1997), 4(1), 45-54

CODEN: GETHEC; ISSN: 0969-7128

PUBLISHER: Stockton

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Effective intracellular expression of small RNA therapeutics depends on a no. of factors. The RNA, whether antisense, **ribozyme**, or RNA aptamer, must be efficiently transcribed, stabilized against rapid degrdn., folded correctly, and directed to the part of the cell where it can be most effective. To overcome a no. of these problems we have been testing expression cassettes based on the human tRNAMet and U6 snRNA promoters, in which transcripts encoding small RNA inserts are protected against attack from the 3' end. Transient expression in cultured cells results in 10<sup>3</sup>-2 .times. 10<sup>7</sup> full-length transcripts per cell, depending partially on the promoter construct used but also on the nature of the insert RNA. 5'-gamma-Phosphate methylation (capping) depended, as expected, on the inclusion of specific U6 snRNA sequences from positions +19 to +27. In situ localization of the transcripts shows that both tRNA and U6 promoter transcripts give primarily punctate nuclear patterns, and that capping of transcripts is not required for nuclear retention. Several different insert RNAs directed against HIV-1 were tested by cotransfection with HIV-1 provirus and assay for subsequent viral reverse transcriptase prodn. These include antisense RNA, hairpin and hammerhead **ribozymes**, and RNA ligands (aptamers) for Tat and Rev RNA-binding proteins. Results show that Rev-binding RNAs efficiently block HIV-1 gene expression, whereas other RNAs have little or no effect

when expressed in these cassettes.

L5 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 6  
ACCESSION NUMBER: 1996:399047 CAPLUS  
DOCUMENT NUMBER: 125:77799  
TITLE: Retroviral vectors designed for target expression of  
RNA polymerase III-driven transcripts: a comparative  
study  
AUTHOR(S): Ilves, Heini; Barske, Carmen; Junker, Uwe; Bohnlein,  
Ernst; Veres, Gabor  
CORPORATE SOURCE: Dep. Mol. Therapy, Systemix Inc., Palo Alto, CA,  
94304, USA  
SOURCE: Gene (1996), 171(2), 203-208  
CODEN: GENED6; ISSN: 0378-1119  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Retroviral gene delivery systems for RNA polymerase II (RNA pol II)-based  
promoters have been developed and are widely used in gene transfer  
studies. In contrast, gene delivery systems with RNA pol III-based  
expression cassettes have not been studied comprehensively, although  
therapeutic applications (e.g., **ribozymes**, antisense, triplex  
RNA and RNA decoys) have been proposed. In this report, we describe  
retroviral vectors designed to optimize expression of short chimeric RNAs  
transcribed from a no. of RNA pol III promoters. Our results show that  
all analyzed RNA pol III expression cassettes (tRNA, **U6**, Ad  
VA1), regardless of orientation, do not transcribe efficiently when  
located between the retroviral long terminal repeats (LTRs). In

contrast,  
high steady-state expression levels can be achieved by inserting the RNA  
pol III expression cassette into the U3 region of the LTR (double-copy  
design). Compared to human tRNA gene promoters (tTMAMet, tRNAVal), the  
human small nuclear RNA **U6** gene (**U6**) and the  
adenovirus virus-assocd. RNA 1 (Ad VA1) gene promoters yielded higher  
expression levels. The majority of the chimeric **U6**-derived  
transcripts were detected in the nuclear RNA fraction, and the VA1 and  
tRNA-driven transcripts were predominantly detected in the cytoplasmic  
compartments. This report is the first comparative study of RNA pol  
III-driven promoters expressing short chimeric transcripts leading to an  
optimized retroviral-vector design.

=> s (ribozyme or antisense) (p) small nuclear

L6 184 (RIBOZYME OR ANTISENSE) (P) SMALL NUCLEAR

=> s l6 and elivery

L7 0 L6 AND ELIVERY

=> s l6 and delivery

L8 15 L6 AND DELIVERY

=> dup rem l8

PROCESSING COMPLETED FOR L8

L9 4 DUP REM L8 (11 DUPLICATES REMOVED)

=> d l- ibib abs

YOU HAVE REQUESTED DATA FROM 4 ANSWERS - CONTINUE? Y/(N):y

L9 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1  
ACCESSION NUMBER: 2000:22565 CAPLUS  
DOCUMENT NUMBER: 133:38719

TITLE: RNA polymerase III-driven expression cassettes in human gene therapy  
AUTHOR(S): Medina, Maria Fe C.; Joshi, Sadhna  
CORPORATE SOURCE: Department of Medical Genetics and Microbiology, Faculty of Medicine, University of Toronto, Toronto, ON, M5S 3E2, Can.  
SOURCE: Curr. Opin. Mol. Ther. (1999), 1(5), 580-594  
CODEN: CUOTFO; ISSN: 1464-8431  
PUBLISHER: Current Drugs Ltd.  
DOCUMENT TYPE: Journal; General Review  
LANGUAGE: English

AB A review, with 87 refs. TRNAs, U6 **small nuclear** RNA and adenovirus virus assocd. RNAs are transcribed by RNA polymerase III. These RNAs are expressed in high levels and possess increased stability due to their small, compact size. Promoters expressing these RNAs have therefore been used to express a variety of interfering RNAs, including ribozymes, **antisense** RNAs and sense or decoy RNAs. Intracellular expression and activity of RNA polymerase III-driven interfering RNAs have been demonstrated in several studies. This review will summarize the design and application of RNA polymerase III-driven expression cassettes in human gene therapy using retroviral vectors as the primary method of gene **delivery**.

REFERENCE COUNT: 63

REFERENCE(S): (1) Adeniyi-Jones, S; Nucleic Acids Res 1984, V12, P1101 CAPLUS  
(2) Arts, G; Curr Biol 1998, V8, P305 CAPLUS  
(3) Arts, G; EMBO J 1998, V17, P7430 CAPLUS  
(4) Baier, G; Mol Immunol 1994, V31, P923 CAPLUS  
(5) Barcellini-Couget, S; Antisense Nucleic Acid Drug Dev 1998, V8, P379 CAPLUS  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2

ACCESSION NUMBER: 1997:257559 CAPLUS

DOCUMENT NUMBER: 126:326162

TITLE: Inhibition of fibrillin 1 expression using U1 snRNA as

a vehicle for the presentation of antisense targeting sequence

AUTHOR(S): Montgomery, Robert A.; Dietz, Harry C.  
CORPORATE SOURCE: Dep. Surgery, Johns Hopkins Univ. Sch. Medicine, Baltimore, MD, 21205, USA

SOURCE: Hum. Mol. Genet. (1997), 6(4), 519-525  
CODEN: HMGE5; ISSN: 0964-6906

PUBLISHER: Oxford University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB This study examines whether the mimicking selected properties of naturally

occurring **antisense** RNAs in prokaryotes allows efficient inhibition of gene expression by in situ-expressed recombinant mols. in mammalian cells. Prokaryotic regulatory transcripts are expressed at

high levels and have hairpin structures at their termini, features reminiscent of **small nuclear** RNAs (snRNAs) which are abundant and stable in the nucleus of all mammalian cells. A sequence complementary

to fibrillin-1 (FBN1) mRNA, interrupted in its center by a hammerhead **ribozyme**, was substituted for the Sm protein binding site between the stem-loop structures of U1 snRNA. Expression of the chimeric **antisense** RNA resulted in dramatic inhibition of expression of fibrillin-1 message and protein in stably transfected cultured cells.

The inhibitory effect was localized to the nucleus. The biol. properties of U1 snRNA may provide a widely applicable vehicle for the in vivo

**delivery of antisense targeting sequences.**

L9 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3  
ACCESSION NUMBER: 1996:399047 CAPLUS  
DOCUMENT NUMBER: 125:77799  
TITLE: Retroviral vectors designed for target expression of  
RNA polymerase III-driven transcripts: a comparative  
study  
AUTHOR(S): Ilves, Heini; Barske, Carmen; Junker, Uwe; Bohnlein,  
Ernst; Veres, Gabor  
CORPORATE SOURCE: Dep. Mol. Therapy, Systemix Inc., Palo Alto, CA,  
94304, USA  
SOURCE: Gene (1996), 171(2), 203-208  
CODEN: GENED6; ISSN: 0378-1119  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Retroviral gene **delivery** systems for RNA polymerase II (RNA pol  
II)-based promoters have been developed and are widely used in gene  
transfer studies. In contrast, gene **delivery** systems with RNA  
pol III-based expression cassettes have not been studied comprehensively,  
although therapeutic applications (e.g., ribozymes, **antisense**,  
triplex RNA and RNA decoys) have been proposed. In this report, we  
describe retroviral vectors designed to optimize expression of short  
chimeric RNAs transcribed from a no. of RNA pol III promoters. Our  
results show that all analyzed RNA pol III expression cassettes (tRNA,

U6,

Ad VAl), regardless of orientation, do not transcribe efficiently when  
located between the retroviral long terminal repeats (LTRs). In

contrast,

high steady-state expression levels can be achieved by inserting the RNA  
pol III expression cassette into the U3 region of the LTR (double-copy  
design). Compared to human tRNA gene promoters (tTMAMet, tRNAVal), the  
human **small nuclear** RNA U6 gene (U6) and the  
adenovirus virus-assocd. RNA 1 (Ad VAl) gene promoters yielded higher  
expression levels. The majority of the chimeric U6-derived transcripts  
were detected in the nuclear RNA fraction, and the VAl and tRNA-driven  
transcripts were predominantly detected in the cytoplasmic compartments.  
This report is the first comparative study of RNA pol III-driven

promoters

expressing short chimeric transcripts leading to an optimized  
retroviral-vector design.

L9 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 4  
ACCESSION NUMBER: 1994:571574 CAPLUS  
DOCUMENT NUMBER: 121:171574  
TITLE: In vivo generation of highly abundant  
sequence-specific oligonucleotides for antisense and  
triplex gene regulation  
AUTHOR(S): Noonberg, Sarah B.; Scott, Gary K.; Garovoy, Marvin  
R.; Benz, Christopher C.; Hunt, C. Anthony  
CORPORATE SOURCE: Bioengineering Graduate Group, Univ. California,  
Berkeley, CA, USA  
SOURCE: Nucleic Acids Res. (1994), 22(14), 2830-6  
CODEN: NARHAD; ISSN: 0305-1048  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB **Antisense** and triplex oligonucleotides continue to demonstrate  
potential as mediators of gene-specific repression of protein synthesis.  
However, inefficient and heterogeneous cellular uptake, intracellular  
sequestration, and rapid intracellular and extracellular degradn.

represent

obstacles to their eventual clin. utility. Efficient cellular  
**delivery** of targeted ribozymes can present similar problems. In  
this report the authors describe a system for circumventing these  
obstacles and producing large quantities of short, sequence-specific RNA  
oligonucleotides for use in these gene regulation strategies. The

oligonucleotides are generated from a vector contg. promoter, capping, and termination sequences from the human **small nuclear U6** gene, surrounding a synthetic sequence incorporating the oligonucleotide of interest. In vivo, these oligonucleotides are produced constitutively and without cell type specificity in levels up to 5.times.10<sup>6</sup> copies per cell, reach steady-state levels of expression with 9 h post-transfection, and are still readily detectable 7 days post-transfection, and are still readily detectable 7 days post-transfection. In addn., these oligonucleotides are retained in the nucleus, obtain a 5' .gamma.-monomethyl phosphate cap, and have an intracellular half-life of approx. on hour. This expression vector provides a novel and efficient method of intracellular **delivery of antisense** or triplex RNA oligonucleotides (and/or ribozymes) for gene regulation, as well as a cost-effective means of comparing the biol. activity arising from a variety of different potential oligonucleotide sequences.

=> d his

(FILE 'HOME' ENTERED AT 10:47:40 ON 03 JAN 2001)

FILE 'CAPLUS, EMBASE, MEDLINE, SCISEARCH' ENTERED AT 10:47:55 ON 03 JAN 2001

```
L1      89 S U6 AND RIBOZYME#
L2      14 S L1 AND LOCALIZ?
L3      5 DUP REM L2 (9 DUPLICATES REMOVED)
L4      24 S L1 AND HIV
L5      8 DUP REM L4 (16 DUPLICATES REMOVED)
L6      184 S (RIBOZYME OR ANTISENSE) (P) SMALL NUCLEAR
L7      0 S L6 AND ELIVERY
L8      15 S L6 AND DELIVERY
L9      4 DUP REM L8 (11 DUPLICATES REMOVED)
```

=> s (ribozyme or antisense) (p) small nucleolar

```
L10     96 (RIBOZYME OR ANTISENSE) (P) SMALL NUCLEOLAR
```

=> s l10 and deliver?

```
L11     2 L10 AND DELIVER?
```

=> dup rem l11

PROCESSING COMPLETED FOR L11

```
L12     2 DUP REM L11 (0 DUPLICATES REMOVED)
```

=> d l- ibib abs

YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y

```
L12 ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2001 ACS
ACCESSION NUMBER:  2000:582845  CAPLUS
TITLE:             Expressing active ribozymes in cells
AUTHOR(S):         Samarsky, Dmitry; Ferbeyre, Gerardo; Bertrand,
Edouard
CORPORATE SOURCE:  HHMI, HHMI/University of Massachusetts Medical
Center,
Worcester, MA, 01605, USA
SOURCE:            Curr. Issues Mol. Biol. (2000), 2(3), 87-93
CODEN: CMBIF6; ISSN: 1467-3037
PUBLISHER:         Caister Academic Press
DOCUMENT TYPE:     Journal
LANGUAGE:          English
```

AB Artificially engineered ribozymes can be used to specifically regulate expression of target genes. Such ribozymes can be synthesized chem. and **delivered** into the cell exogeneously. Alternatively, ribozymes can be produced by the cell endogenously, after introduction of the artificial gene into the cellular genome. In the latter case, the design of the artificial gene defines the **ribozyme** properties, such as: expression level, intracellular localization, folding and assocn. with proteins. Generally speaking, design of the expression vector is crit.

to

obtain active **ribozyme** mols. This paper first describes factors that are known or predicted to affect **ribozyme** activity in the cell, then reviews various expression systems that have been specifically developed for ribozymes. Lastly, a recently developed **ribozyme** system termed snorbozymes (**small nucleolar RNA:ribozyme** hybrids) will be discussed. This powerful test system has generated several important observations that are likely to affect

the

future development of **ribozyme** technol.

REFERENCE COUNT: 39

REFERENCE(S): (1) Bachellerie, J; Trends Biochem Sci 1997, V22, P257

CAPLUS

(2) Bertrand, E; EMBO J 1994, V13, P2904 CAPLUS

(3) Bertrand, E; Nucl Acids Res 1994, V22, P293

CAPLUS

(4) Bertrand, E; RNA 1997, V3, P75 CAPLUS

(5) Cagnon, L; J Acquir Immune Defic Syndr Hum Retrovirol 1995, V9, P349 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:718538 CAPLUS

DOCUMENT NUMBER: 132:189158

TITLE: Expressing active ribozymes in cells

AUTHOR(S): Samarsky, Dmitry; Ferbeyre, Gerardo; Bertrand, Edouard

CORPORATE SOURCE: Medical Center Program in Molecular Medicine, HHMI/University of Massachusetts, Worcester, MA, 01605, USA

SOURCE: Intracell. Ribozyme Appl. (1999), 79-91. Editor(s): Rossi, John J.; Couture, Larry A. Horizon Scientific Press: Norfolk, UK.

CODEN: 68IWAY

DOCUMENT TYPE: Conference; General Review

LANGUAGE: English

AB A review with 38 refs. Artificially engineered ribozymes can be used to specifically regulate expression of target genes. Such ribozymes can be synthesized chem. and **delivered** into the cell exogenously. Alternatively, ribozymes can be produced by the cell endogenously, after introduction of the artificial gene into the cellular genome. In the latter case, the design of the artificial gene defines the **ribozyme** properties, such as: expression level, intracellular localization, folding and assocn. with proteins. Generally speaking, design of the expression vector is crit. to obtain active **ribozyme** mols. This paper first describes factors that are known or predicted to affect **ribozyme** activity in the cell, then reviews various expression systems that have been specifically developed for ribozymes. Lastly, a recently developed **ribozyme** system termed snorbozymes (**small nucleolar RNA:ribozyme** hybrids) will be discussed. This powerful test system has generated several important observations that are likely to affect the future development of **ribozyme** technol.

REFERENCE COUNT: 38

REFERENCE(S): (1) Bachellerie, J; Trends Biochem Sci 1997, V22, P257

CAPLUS

(2) Bertrand, E; EMBO J 1994, V13, P2904 CAPLUS  
 (3) Bertrand, E; Nucl Acids Res 1994, V22, P293  
 CAPLUS  
 (4) Bertrand, E; RNA 1997, V3, P75 CAPLUS  
 (5) Cagnon, L; J Acquir Immune Defic Syndr Hum  
 Retrovirol 1995, V9, P349 CAPLUS  
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s (ribozyme# or antisense) and trna  
 L13 1082 (RIBOZYME# OR ANTISENSE) AND TRNA  
 => s l13 and locali?  
 L14 54 L13 AND LOCALI?  
 => s l14 and inhibit?  
 L15 24 L14 AND INHIBIT?  
 => dup rem l15  
 PROCESSING COMPLETED FOR L15  
 L16 11 DUP REM L15 (13 DUPLICATES REMOVED)  
 => d l- ibib abs  
 YOU HAVE REQUESTED DATA FROM 11 ANSWERS - CONTINUE? Y/(N):y

L16 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1  
 ACCESSION NUMBER: 1998:382462 CAPLUS  
 DOCUMENT NUMBER: 129:106784  
 TITLE: Selective disruption of neuregulin-1 function in  
 vertebrate embryos using **ribozyme-**  
**tRNA** transgenes  
 AUTHOR(S): Zhao, Jack Jiagang; Lemke, Greg  
 CORPORATE SOURCE: Molecular Neurobiology Laboratory, Salk Institute for  
 Biological Studies, La Jolla, CA, 92037, USA  
 SOURCE: Development (Cambridge, U. K.) (1998), 125(10),  
 1899-1907  
 CODEN: DEVPED; ISSN: 0950-1991  
 PUBLISHER: Company of Biologists Ltd.  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB The products of the neuregulin-1 gene constitute a set of polypeptide  
 growth factors whose signaling through the ErbB receptors is essential to  
 the growth and differentiation of many cell types in culture. Although  
 studies with neuregulin-1 mutant mice have demonstrated that these growth  
 factors are also essential regulators of cellular differentiation in  
 vivo,  
 the mid-embryonic death of these mutants precludes an anal. of  
 hypothesized neuregulin-1 roles in later aspects. of development. To  
 circumvent this early lethality, the authors have pursued a  
**ribozyme**-based strategy for the perturbation of neuregulin-1  
 function in developing chick embryos. Early administration of a  
 retrovirus carrying neuregulin-1 hammerhead-type **ribozymes** to  
 blastoderm-stage embryos leads to an embryonic lethal phenotype that  
 results from the failure of ventricular trabeculation in the developing  
 heart, a faithful phenocopy of the mouse neuregulin-1 mutations. Later,  
 more **localized** delivery of the **ribozyme** to the  
 developing retina **inhibits** both the differentiation of retinal  
 ganglion cell neurons and the proliferation of the neuroepithelial cells  
 from which they derive. These results suggest that neuregulin-1 promotes  
 both muscle cell differentiation in the heart and neuronal  
 differentiation

in the central nervous system. In addn., they demonstrate the utility of hammerhead **ribozymes** as a simple, effective and easily adaptable method of conditional gene inactivation in vertebrates.

L16 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:667263 CAPLUS  
DOCUMENT NUMBER: 127:322794  
TITLE: Property-affecting and/or property-exhibiting compositions for therapeutic and diagnostic uses  
INVENTOR(S): Rabbani, Elazar; Stavrianopoulos, Jannis G.; Donegan, James J.; Liu, Dakai; Kelker, Norman E.; Engelhardt, Dean L.  
PATENT ASSIGNEE(S): Enzo Therapeutics, Inc., USA  
SOURCE: Can. Pat. Appl., 275 pp.  
CODEN: CPXXEB  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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CA 2190304	AA	19970616	CA 1996-2190304	19961114
EP 779365	A2	19970618	EP 1996-119961	19961212
EP 779365	A3	19991124		
R: DE, FR, GB, IT				
JP 09313190	A2	19971209	JP 1996-360043	19961216
PRIORITY APPLN. INFO.:			US 1995-574443	19951215

AB Compsns. useful for effecting and/or exhibiting changes in biol. functioning and processing in cells and biol. systems are provided which combine chem. modifications and/or ligand addns. with biol. functions in such a way as not to interfere substantially with the biol. functions. Such addnl. characteristics include nuclease resistance, targeting specific cells or cell receptors, and augmenting or decreasing interactions between the compsns. and target cells. A title compn. may constitute a nucleotide, nucleotide analog, nucleic acid, natural or synthetic polymer, ligand, or conjugate of a ligand with any of the preceding. For example, single-stranded DNA from a plasmid contg. a gene of interest is complexed with an allylamine phosphoramidite-contg. oligonucleotide primer (complementary to a region of the DNA distant from the gene of interest) which has been modified with trilactosyllslysine (prepn. given), and the primer is extended with Klenow enzyme to form completely double-stranded DNA. On exposure of target cells to this DNA, the galactose moieties on the DNA bind to receptors on the cells, resulting in transport of the DNA into the cells. In another embodiment, DNA for **antisense** RNA sequences to regions of the HIV genome were inserted into the U1 small nuclear RNA coding region and the DNA was used to transform U937 cells. The transformed cells were resistant to

HIV infection, as shown by **inhibition** of virus replication and p24 antigen prodn.

L16 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2001 ACS

DUPLICATE 2

ACCESSION NUMBER: 1997:554864 CAPLUS  
DOCUMENT NUMBER: 127:215663  
TITLE: Yeast 5-aminolevulinate synthase provides additional chlorophyll precursor in transgenic tobacco  
AUTHOR(S): Zavgorodnyaya, Anna; Papenbrock, Jutta; Grimm, Bernhard  
CORPORATE SOURCE: Institute of Plant Genetics and Crop Plant Research, Gatersleben, 06466, Germany  
SOURCE: Plant J. (1997), 12(1), 169-178  
CODEN: PLJUED; ISSN: 0960-7412  
PUBLISHER: Blackwell  
DOCUMENT TYPE: Journal  
LANGUAGE: English



AB Synthesis of the tetrapyrrole precursor 5-aminolevulinate (ALA) in plants starts with glutamate and is a **tRNA**-dependent pathway consisting of three enzymic steps **localized** in plastids. In animals and yeast, ALA is formed in a single step from succinyl CoA and glycine by aminolevulinate synthase (ALA-S) in mitochondria. A gene encoding a fusion protein of yeast ALA-S with an aminoterminal transit sequence for the small subunit of ribulose biphosphate carboxylase was introduced into the genome of wild-type tobacco and a chlorophyll-deficient transgenic line expressing glutamate 1-semi-aldehyde aminotransferase (GSA-AT) **antisense** RNA. Expression of ALA-S in the GSA-AT **antisense** transgenic line provided green-pigmented co-transformants similar to wild-type in chlorophyll content, while transformants derived from wild-type plants did not show phenotypical changes. The capacity to synthesize ALA and chlorophyll was increased in transformed plants, indicating a contribution of ALA-S to the ALA supply for chlorophyll synthesis. ALA-S activity was detected in plastids of the transformants. Preliminary evidence is presented that succinyl CoA, the substrate for ALA-S, can be synthesized and metabolized in plastids. The transgenic plants formed chlorophyll in the presence of gabaculine, an **inhibitor** of GSA-AT. Steady-state RNA and protein levels and, consequently, the enzyme activity of GSA-AT were reduced in plants expressing ALA-S. In analogy to the light-dependent ALA synthesis attributed to feedback regulation, a mechanism at the level of intermediates or tetrapyrrole end-products is proposed, which coordinates the need for heme and chlorophyll precursors and restricts synthesis of ALA by regulating GSA-AT gene expression. The genetically engineered tobacco plants contg. the yeast ALA-S activity demonstrate functional complementation of the catalytic activity of the plant ALA-synthesizing pathway and open strategies for producing tolerance against **inhibitors** of the C5 pathway.

L16 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3  
 ACCESSION NUMBER: 1997:452049 CAPLUS  
 DOCUMENT NUMBER: 127:174530  
 TITLE: Role of the glycolytic protein, glyceraldehyde-3-phosphate dehydrogenase, in normal cell function and in cell pathology  
 AUTHOR(S): Sirover, Michael A.  
 CORPORATE SOURCE: Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA, 19140, USA  
 SOURCE: J. Cell. Biochem. (1997), 66(2), 133-140  
 CODEN: JCEBD5; ISSN: 0730-2312  
 PUBLISHER: Wiley-Liss  
 DOCUMENT TYPE: Journal; General Review  
 LANGUAGE: English

AB A review with .apprx.40 refs. The glycolytic protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH) appeared to be an archetypical protein of limited excitement. However, independent studies from a no. of different labs. reported a variety of diverse biol. properties of the GAPDH protein.

As a membrane protein, GAPDH functions in endocytosis; in the cytoplasm, it is involved in the translational control of gene expression; in the nucleus, it functions in nuclear **tRNA** export, in DNA replication, and in DNA repair. The intracellular **localization** of GAPDH may be dependent on the proliferative state of the cell. Recent studies identified a role for GAPDH in neuronal apoptosis. GAPDH gene expression was specifically increased during programmed neuronal cell death. Transfection of neuronal cells with **antisense** GAPDH sequences **inhibited** apoptosis. Lastly, GAPDH may be directly involved in the cellular phenotype of human neurodegenerative disorders, esp. those characterized at the mol. level by the expansion of CAG repeats. In this review, the current status of ongoing GAPDH studies are described (with the exception of its unique oxidative modification by nitric oxide). Consideration of future directions are suggested.

L16 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 4  
 ACCESSION NUMBER: 1997:46429 CAPLUS  
 DOCUMENT NUMBER: 126:71160  
 TITLE: The expression cassette determines the functional activity of **ribozymes** in mammalian cells by controlling their intracellular **localization**  
 AUTHOR(S): Bertrand, Edouard; Castanotto, Daniela; Zhou, Chen; Carbonnelle, Cecile; Lee, Nan Sook; Good, Paul; Chatterjee, Saswati; Grange, Thierry; Pictet, Raymond;  
 Kohn, Donald; Engelke, David; Rossi, John J.  
 CORPORATE SOURCE: Dep. Molecular Biol., Beckman Research Institute City of Hope, Duarte, CA, 91010, USA  
 SOURCE: RNA (1997), 3(1), 75-88  
 CODEN: RNARFU; ISSN: 1355-8382  
 PUBLISHER: Cambridge University Press  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB In order to better understand the influence of RNA transcript context on RNA **localization** and catalytic RNA efficacy in vivo, we have constructed and characterized several expression cassettes useful for transcribing short RNAs with well defined 5' and 3' appended flanking sequences. These cassettes contain promoter sequences from the human U1 snRNA, U6 snRNA, or tRNAMet genes, fused to various processing/stabilizing sequences. The levels of expression and the sub-cellular **localization** of the resulting RNAs were detd. and compared with those obtained from Pol II promoters normally linked to mRNA prodn., which include a cap and polyadenylation signal. The tRNA, U1, and U6 transcripts were nuclear in **localization** and expressed at the highest levels, while the std. Pol II promoted transcripts were cytoplasmic and present at lower levels. The ability of these cassettes to confer **ribozyme** activity in vivo was tested with two assays. First, an SIV-growth hormone reporter gene was transiently transfected into human embryonic kidney cells expressing an anti-SIV **ribozyme**. Second, cultured T lymphocytes expressing an anti-HIV **ribozyme** were challenged with HIV. In both cases, the **ribozymes** were effective only when expressed as capped, polyadenylated RNAs transcribed from Pol II cassettes that generate a cytoplasmically **localized ribozyme** that facilitates co-**localization** with its target. The inability of the other cassettes to support **ribozyme-mediated inhibitory** activity against their cytoplasmic target is very likely due to the resulting nuclear **localization** of these **ribozymes**. Thus, the **ribozyme** expression cassette detcs. its intracellular **localization** and, hence, its corresponding functional activity.

L16 ANSWER 6 OF 11 SCISEARCH COPYRIGHT 2001 ISI (R)  
 ACCESSION NUMBER: 97:103243 SCISEARCH  
 THE GENUINE ARTICLE: WE183  
 TITLE: Expression of small, therapeutic RNAs in human cell nuclei  
 AUTHOR: Good P D; Krikos A J; Li S X L; Bertrand E; Lee N S; Giver L; Ellington A; Zaia J A; Rossi J J; Engelke D R  
 (Reprint)  
 CORPORATE SOURCE: UNIV MICHIGAN, DEPT BIOL CHEM, 1301 CATHERINE RD, ANN ARBOR, MI 48109 (Reprint); UNIV MICHIGAN, DEPT BIOL CHEM, ANN ARBOR, MI 48109; CITY HOPE NATL MED CTR, DEPT PEDIAT, DUARTE, CA 91010; LOMA LINDA UNIV, CTR MOL BIOL & GENE TECHNOL, LOMA LINDA, CA 92350; INDIANA UNIV, DEPT CHEM, BLOOMINGTON, IN  
 COUNTRY OF AUTHOR: USA  
 SOURCE: GENE THERAPY, (JAN 1997) Vol. 4, No. 1, pp. 45-54.  
 Publisher: STOCKTON PRESS, HOUNDMILLS, BASINGSTOKE,

DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: LIFE  
 LANGUAGE: English  
 REFERENCE COUNT: 42

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Effective intracellular expression of small RNA therapeutics depends on

a number of factors. The RNA, whether **antisense**, **ribozyme**, or RNA aptamer, must be efficiently transcribed, stabilized against rapid degradation, folded correctly, and directed to the part of the cell where it can be most effective. To overcome a number of these problems we have been testing expression cassettes based on the human **tRNA**(met) and U6 snRNA promoters, in which transcripts encoding small RNA inserts are protected against attack from the 3' end. Transient expression in cultured cells results in  $10(3)-2 \times 10(7)$  full-length transcripts per cell, depending partially on the promoter construct used but also on the nature of the insert RNA. 5' gamma-Phosphate methylation (capping) depended, as expected, on the inclusion of specific U6 snRNA sequences from positions +19 to +27. **in situ localization** of the transcripts shows that both **tRNA** and U6 promoter transcripts give primarily punctate nuclear patterns, and that capping of transcripts is not required for nuclear retention. Several different insert RNAs directed against HIV-1 were tested by cotransfection with HIV-1 provirus and assay for subsequent viral reverse transcriptase production. These include **antisense** RNA, hairpin and hammerhead **ribozymes**, and RNA ligands (aptamers) for Tat and Rev RNA binding proteins. Results show that Rev-binding RNAs efficiently block HIV-1 gene expression, whereas other RNAs have little or no effect when expressed in these cassettes.

L16 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:869519 CAPLUS  
 DOCUMENT NUMBER: 123:275993  
 TITLE: HIV-1 virus tRNA<sup>Lys</sup>-**ribozyme** and use for lentivirus vaccine or anti-viral agent  
 INVENTOR(S): Rossi, John J.; Larson, Garry P.  
 PATENT ASSIGNEE(S): City of Hope, USA  
 SOURCE: PCT Int. Appl., 43 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 2  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9519788	A1	19950727	WO 1994-US13798	19941202
W: AU, CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5827935	A	19981027	US 1994-185827	19940124
CA 2157015	AA	19950727	CA 1994-2157015	19941202
AU 9513335	A1	19950808	AU 1995-13335	19941202
AU 692208	B2	19980604		
EP 707493	A1	19960424	EP 1995-904785	19941202
R: DE, FR, GB				
PRIORITY APPLN. INFO.:			US 1994-185827	19940124
			WO 1992-US4362	19920527
			WO 1994-US13798	19941202

AB The invention provides mechanisms for the co-localization in a living cell of a target mol. and of an **inhibitor** for the target mol. The invention also provides novel chimeric tRNA<sup>Lys</sup>-**ribozyme** mols. that compete effectively with tRNA<sup>Lys</sup> for HIV-1 reverse transcriptase binding site. The chimeric human tRNA<sup>Lys</sup>-**ribozymes** **inhibit** reverse HIV transcription by delivering **inhibitors**

such as **ribozymes** of HIV-1 reverse transcriptase directly to the virion particle and render it non-functional. The chimeric mols. of the invention thus serve as highly specific non-toxic therapeutic agents and vaccines for viral, including lentiviral, infections. These chimeric mols. also reveal a novel, site specific RNA cleaving activity of HIV-1.

L16 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:878130 CAPLUS

DOCUMENT NUMBER: 123:306062

TITLE: A chimeric tRNA<sup>Lys3</sup>-**ribozyme**  
**inhibits** HIV replication following virion assembly

AUTHOR(S): Westaway, Shawn K.; Larson, Garry P.; Li, Shirley; Zaia, John A.; Rossi, John J.

CORPORATE SOURCE: Center Mol. Biology Gene Therapy, Loma Linda Univ., Loma Linda, CA, 92350, USA

SOURCE: Nucleic Acids Symp. Ser. (1995), 33(Symposium on RNA Biology, I. RNA-Protein Interactions, 1995), 194-9  
CODEN: NACSD8; ISSN: 0261-3166

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Co-localization of **ribozymes** with their appropriate target is one method utilized to increase their effectiveness in vivo. Effective antiviral **ribozymes** will likely rely on mechanisms which direct the **ribozyme** to the genomic or subgenomic RNAs. Exploiting the fact that a specific host cellular tRNA primer is bound by viral proteins and co-packaged with viral genomes in newly synthesized virions, **ribozymes** were fused to the 3'-terminus of tRNA<sup>Lys3</sup> to direct their activity to cleave the HIV-1 genome. This chimeric **ribozyme** is catalytically active in vitro, and is efficiently recognized and bound by HIV-1 reverse transcriptase with affinities similar to tRNA<sup>Lys3</sup>. The intragenic RNA polymerase III promoter entity of the tRNA allows for high levels of expression of the tRNA-RBZ and the preferential localization of transcript within the cytoplasm in transfected cells. This **ribozyme** was effective in reducing the infectivity of a viral stock which was produced from transiently transfected cells bearing the chimeric gene. These results demonstrate the feasibility of using tRNAs as a means of co-localizing **ribozymes** with their viral genomic RNA targets. The possibility exists to fuse stable RNAs to **ribozymes** as a means of increasing their stability and localizing them to their appropriate target sites.

L16 ANSWER 9 OF 11 MEDLINE

ACCESSION NUMBER: 96219298 MEDLINE

DOCUMENT NUMBER: 96219298

TITLE: A chimeric tRNA(Lys3)-**ribozyme**  
**inhibits** HIV replication following virion assembly.

AUTHOR: Westaway S K; Larson G P; Li S; Zaia J A; Rossi J J

CORPORATE SOURCE: Center for Molecular Biology and Gene Therapy, Loma Linda University, CA 92350, USA.

CONTRACT NUMBER: AI25959 (NIAID)

AI29329 (NIAID)

SOURCE: NUCLEIC ACIDS SYMPOSIUM SERIES, (1995) (33) 194-9.

Journal code: O8N. ISSN: 0261-3166.

PUB. COUNTRY: ENGLAND: United Kingdom

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199609

AB Co-localization of **ribozymes** with their appropriate target is one method utilized to increase their effectiveness in vivo. Effective antiviral **ribozymes** will likely rely on mechanisms which direct the **ribozyme** to the genomic or subgenomic RNAs. Exploiting the fact that a specific host cellular tRNA primer is bound by viral proteins and co-packaged with viral genomes in newly

synthesized virions, **ribozymes** were fused to the 3'-terminus of **tRNA**(Lys3) in an attempt to direct their activity to cleave the HIV-1 genome. This chimeric **ribozyme** is catalytically active in vitro, and is efficiently recognized and bound by HIV-1 reverse transcriptase with affinities similar to **tRNA**(Lys3). The intragenic RNA polymerase III promoter entity of the **tRNA** allows for high levels of expression of the **tRNA**-RBZ and the preferential **localization** of transcript within the cytoplasm in transfected cells. This **ribozyme** was effective in reducing the infectivity of a viral stock which was produced from transiently transfected cells bearing the chimeric gene. These results demonstrate the feasibility of using tRNAs as a means of co-localizing **ribozymes** with their viral genomic RNA targets. The possibility exists to fuse stable RNAs to **ribozymes** as a means of increasing their stability and **localizing** them to their appropriate target sites.

L16 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2001 ACS  
 ACCESSION NUMBER: 1990:212940 CAPLUS  
 DOCUMENT NUMBER: 112:212940  
 TITLE: The methylation of one specific guanosine in a pre-**tRNA** prevents cleavage by RNase P and by the catalytic M1 RNA  
 AUTHOR(S): Kahle, Dietmar; Wehmeyer, Uta; Char, Shoba; Krupp, Guido  
 CORPORATE SOURCE: Inst. Gen. Microbiol., Christian-Albrechts Univ., Kiel, D-2300, Fed. Rep. Ger.  
 SOURCE: Nucleic Acids Res. (1990), 18(4), 837-44  
 CODEN: NARHAD; ISSN: 0305-1048  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB Several modified nucleosides were introduced during in vitro RNA synthesis into a pre-tRNAs<sup>er</sup>. The pre-tRNAs were used as substrates for RNase P enzymes. No effects were obsd. with biotin-8-ATP or [ $\alpha$ .-G]-GTP, whereas with m7GTP (where m7G is 7-methylguanosine), the cleavage reaction was completely **inhibited**. Anal. of pre-tRNAs which contained m7G at various positions revealed a single base at the 5'-end of the acceptor stem where this modification absolutely prevents cleavage by catalytic M1 RNA, eukaryotic and prokaryotic RNase P holoenzymes. These results suggest that a crit. contact must be made between pre-tRNA substrate and enzyme/**ribozyme** or that the approach of the potential cleaving agent (a pos. Mg ion) is made impossible by the pos. charge at N-7 of the guanosine. In addn., it was shown that a pre-tRNA contg. only m7Gs can still form a complex with M1 RNA in a gel retardation assay.

L16 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 5  
 ACCESSION NUMBER: 1990:52942 CAPLUS  
 DOCUMENT NUMBER: 112:52942  
 TITLE: **Ribozyme** mediated destruction of RNA in vivo  
 AUTHOR(S): Cotten, Matt; Birnstiel, Max L.  
 CORPORATE SOURCE: Res. Inst. Mol. Pathol., Vienna, A-1030, Austria  
 SOURCE: EMBO J. (1989), 8(12), 3861-6  
 CODEN: EMJODG; ISSN: 0261-4189  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB Previous studies have demonstrated that high **ribozyme** to substrate ratios are required for **ribozyme** inhibitory function in nuclear exts. To obtain high intracellular levels of **ribozymes**, **tRNA** genes, known to be highly expressed in most tissues, have been modified for use as **ribozyme** expression cassettes. **Ribozyme** coding sequences were placed between the A and the B box, internal promoter sequences of a Xenopus tRNAMet gene.

When injected into the nucleus of frog oocytes, the **ribozyme** **trRNA** gene (ribtDNA) produces hammerhead **ribozymes** which cleave the 5' sequences of U7snRNA, its target substrate, with high efficiency in vitro. Oocytes were coinjected with ribtDNA, U7snRNA and control substrate RNA devoid of a cleavage sequence. The ribtRNA remained **localized** mainly in the nucleus, whereas the substrate and the control RNA exited rapidly into the cytoplasm. However, sufficient ribtRNA migrated into the cytoplasm to cleave, and destroy, the U7snRNA. Thus, the action of targeted hammerhead **ribozymes** in vivo is demonstrated.

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L4 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1  
ACCESSION NUMBER: 1992:503626 CAPLUS  
DOCUMENT NUMBER: 117:103626  
TITLE: Overexpression of RRE-derived sequences inhibits  
HIV-1 replication in CEM cells  
AUTHOR(S): Lee, Thomas C.; **Sullenger, Bruce A.**;  
Gallardo, Humilidad F.; Ungers, Grace E.; Gilboa, Eli  
CORPORATE SOURCE: Sloan-Kettering Inst., New York, NY, 10021, USA  
SOURCE: New Biol. (1992), 4(1), 66-74  
CODEN: NEBIE2; ISSN: 1043-4674  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Overexpression of sequences corresponding to the major Rev-binding site  
in the Rev response element of human immunodeficiency virus type 1 (HIV-1)  
(RRE decoys) was used to render cells resistant to HIV-1 replication.  
This was accomplished by the use of a chimeric **tRNA**-RRE  
transcription unit in a double-copy murine retroviral vector to express  
high levels of HIV-1 RRE-contg. transcripts in CEM SS cells. Replication  
of HIV-1 was inhibited more than 90% in cells expressing chimeric  
**tRNA**-RRE transcripts, as detd. by in situ immunofluorescence anal.  
and a p24 antigen ELISA test. Anal. of RNA from HIV-1-infected cells  
suggests that expression of RRE-contg. sequences in CEM SS cells inhibits  
HIV-1 replication by interfering with Rev function, presumably by  
competing for Rev binding to its physiol. target. The use of a  
subfragment of RRE as decoy RNA reduces the likelihood that essential  
cellular factors will be sequestered in cells expressing the decoy RNA.  
Thus, use of RRE-based decoy RNA to inhibit HIV-1 replication may  
represent a safer alternative to the use of TAR decoy RNA.

L4 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2001 ACS  
ACCESSION NUMBER: 1993:117747 CAPLUS  
DOCUMENT NUMBER: 118:117747  
TITLE: Development of a **tRNA** based transcription  
system to render cells resistant to viral replication  
AUTHOR(S): **Sullenger, Bruce Alan**  
CORPORATE SOURCE: Med. Coll., Cornell Univ., New York, NY, USA  
SOURCE: (1991) 220 pp. Avail.: Univ. Microfilms Int., Order  
No. DA9135421  
From: Diss. Abstr. Int. B 1992, 52(7), 3444  
DOCUMENT TYPE: Dissertation  
LANGUAGE: English  
AB Unavailable

L4 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2001 ACS  
ACCESSION NUMBER: 1991:242041 CAPLUS  
DOCUMENT NUMBER: 114:242041  
TITLE: Stably transformed eucaryotic cells expressing a  
foreign gene from an RNA polymerase III promoter  
INVENTOR(S): Gilboa, Eli; **Sullenger, Bruce**  
PATENT ASSIGNEE(S): Sloan-Kettering Institute for Cancer Research, USA  
SOURCE: PCT Int. Appl., 76 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent

drewwang

LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9013641	A1	19901115	WO 1990-US2656	19900510
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, IT, LU, NL, SE				
EP 471796	A1	19920226	EP 1990-909233	19900510
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE				
JP 04505261	T2	19920917	JP 1990-508724	19900510
PRIORITY APPLN. INFO.:			US 1989-354171	19890510
			WO 1990-US2656	19900510

AB Retroviral vectors contg. a heterologous gene expressed from an RNA polymerase III promoter, and stably transformed eukaryotic cells prepd. with these vectors are described. These vectors may be used in vaccines, e.g. against HIV. A retroviral vector based on Moloney murine leukemia virus was prepd. A truncated **tRNA** gene fused to antisense sequences complementary to parts of the HIV-1 genome was cloned into the 3' LTR of such a vector. Replication of HIV-1 in HUT78 cells contg. this vector was inhibited.

L4 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2  
ACCESSION NUMBER: 1991:75955 CAPLUS  
DOCUMENT NUMBER: 114:75955  
TITLE: Expression of chimeric **tRNA**-driven antisense transcripts renders NIH 3T3 cells highly resistant to Moloney murine leukemia virus replication  
AUTHOR(S): **Sullenger, Bruce A.**; Lee, Thomas C.; Smith, Clayton A.; Ungers, Grace E.; Gilboa, Eli  
CORPORATE SOURCE: Bone Marrow Transplant Serv., Mem. Sloan-Kettering Cancer Cent., New York, NY, 10021, USA  
SOURCE: Mol. Cell. Biol. (1990), 10(12), 6512-23  
CODEN: MCEBD4; ISSN: 0270-7306  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB NIH 3T3 cells infected with Moloney murine leukemia virus (MoMLV) express high levels of virus-specific RNA. To inhibit replication of the virus, chimeric **tRNA** genes encoding antisense templates were stably introduced into NIH 3T3 cells via a retroviral vector. Efficient expression of hybrid **tRNA**-MoMLV antisense transcripts and inhibition of MoMLV replication were dependent on the use of a particular type of retroviral vector, the double-copy vector, in which the chimeric **tRNA** gene was inserted in the 3' long terminal repeat. MoMLV replication was inhibited up to 97% in cells expressing antisense RNA corresponding to the gag gene and less than 2-fold in cells expressing antisense RNA corresponding to the pol gene. RNA and protein analyses suggest that inhibition was exerted at the level of translation. These results suggest that RNA polymerase III-based antisense inhibition systems

can be used to inhibit highly expressed viral genes and render cells resistant to viral replication via intracellular immunization strategies.

L4 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3  
ACCESSION NUMBER: 1991:18251 CAPLUS



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DOCUMENT NUMBER: 114:18251  
TITLE: Overexpression of TAR sequences renders cells  
resistant to human immunodeficiency virus replication  
AUTHOR(S): Sullenger, Bruce A.; Gallardo, Humilidad F.;  
Ungers, Grace E.; Gilboa, Eli  
CORPORATE SOURCE: Program Mol. Biol., Mem. Sloan-Kettering Cancer  
Cent.,  
New York, NY, 10021, USA  
SOURCE: Cell (Cambridge, Mass.) (1990), 63(3), 601-8  
CODEN: CELLB5; ISSN: 0092-8674  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Overexpression of TAR-contg. sequences (TAR decoys) was used to render  
cells resistant to HIV replication. A chimeric tRNA<sup>met</sup>-TAR  
transcription

unit contained in a double copy murine retroviral vector was used to  
express high levels of HIV-1 TAR-contg. transcripts in CEM SS cells.  
Replication of HIV-1 was inhibited over 99% in cells expressing chimeric  
tRNA-TAR transcripts, but an amphotropic murine retrovirus  
replicated normally in these cells. Expression of TAR sequences in CEM

SS  
cells had no adverse effects on cell viability, indicating that essential  
cellular factors are not being sequestered in these cells. TAR decoy  
RNA-mediated HIV inhibition may also be effective against natural HIV  
isolates in spite of their hypervariable nature, as suggested by the fact  
that replication of SIVmac was also inhibited in cells expressing HIV-1  
TAR decoys.

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